Horses and cows might teach us about human knees.

Holland, C.[1, 2], Vollrath, F. [1]* and Gill, H.S. [3,4]

[1] Department of Zoology, University of Oxford, South Parks Road, Oxford, UK

[2] Department of Materials Science and Engineering, University of Sheffield, Mappin Street, Sheffield, UK.

[3] Nuffield Department of Orthopaedics, Rheumatology and Musculoskeletal Sciences, University of Oxford, Nuffield Orthopaedic Centre, Oxford, UK.

[4] Department of Mechanical Engineering, University of Bath, Claverton Down, Bath, UK Bath, UK

* corresponding author : Prof. Fritz Vollrath,

Department of Zoology, South Parks Road, Oxford, Oxfordshire, OX1 3PS, UK

Tel: +44 (0) 1865 271 216, Email: fritz.vollrath@zoo.ox.ac.uk

Abstract

Our comparative study of the knees of horses and cows (paraphrased as highly evolved joggers and as domesticated couch-potatoes, respectively) demonstrates significant differences in the posterior sections of bovine and equine tibial cartilage, which are consistent with specialization for gait. These insights were possible using a novel analytical measuring technique based on the shearing of small biopsy samples, called Dynamic Shear Analysis. We assert that this technique could provide a powerful new tool to precisely quantify the pathology of osteoarthritis for the medical field.

Keywords

Dynamic-Shear-Analysis, osteoarthritis, cartilage

Introduction

Millennia of natural selection have shaped the form and function of animal joints. Of special importance is a joint's response to loading and the distribution of stresses and strains across its surfaces. Hence it is of significant interest both academically and practically to understand, firstly, how animals have co-evolved and integrated morphology, materials and mobility of specific joints and, secondly, how these structural and functional features are further integrated into behaviour i.e. gaits. When such highly evolved systems fail there is subsequent cost; in Nature lameness reduces physical fitness and thus increases the chance of predation and death, for man joint diseases represent individual pain and disability(Desmeules et al. 2010; Muraki et al. 2010) as well as significant socioeconomic drain(Gupta et al. 2005). There are feedback links between morphology and gait; with, for example, decades of un-natural impact (for example jogging with wrong shoes and foot-fall) beginning to leave their legacy in the traumatic wear and tear of human knees(Felson et al. 1997). Here we propose that a comparative study of the knees of horses and cows (paraphrased as highly evolved joggers and as domesticated couch-potatoes, respectively) might allow us to test specific as well as generic hypotheses about the form and function of integrated knee morphology and cartilage.

For human knees an important failure mode is osteoarthritis (OA), which is the result of degradation of the articular cartilage within the joint and often begins in specific locations(Gulati et al. 2009). The role of the cartilage is to provide a low-friction, self lubricating bearing surface transmitting loads and permitting movement. It is primarily composed of a tightly ordered network of type II collagen fibres embedded in a highly hydrated matrix of proteoglycans. Whilst much is known about the biochemistry and genetics of this biological composite, relatively little is known of how these components contribute to the bulk material properties and if/how they are optimised within a joint. However, characterising the mechanical properties of cartilage across the surface of the tibia is not trivial. Typically this has required either (i) the excision of significant amounts of tissue to allow for the curvature of the underlying bony surface or (ii) the use of cumbersome equipment (Appleyard et al. 2003; Young et al. 2007).

Hence, there is a clear need to develop a simple technique that will allow the testing of small tissue samples at physiological conditions. Here we present one such technique, Dynamic Shear Analysis (DSA) and its suitability for the study of small biopsy samples of knee cartilage. DSA employs rheometry, which gives us detailed measurements of the behaviour of a material in response to highly controlled shear forces (Chaudhury et al. 2011). Specifically, DSA examines material properties by compressing a sample with a known force between two metal plates. The sample is then oscillated parallel to the plates at an extremely small fixed strain over a range of frequencies whilst measuring the resistance to this deformation. The dynamic information provided (the shear modulus, G*) from such a test is indicative of the overall integrity of the collagen network . Thus DSA allows us to easily and quickly examine in great detail the force response curves of small *ex vivo* samples in body fluids and at body temperatures.

We chose the ‘knees’ of horses and cows for comparison in order to test the hypothesis that there are correlations between the lifestyle of an animal (mobility, gait) and the form and function of its knees, with our uncertainty whether the differences are more in the details of knee morphology or more in the details of cartilage material properties.

Methods

Sample preparation

Bovine and equine specimens were obtained through dissection of full intact knees from cadaverous material obtained from a slaughterhouse. A pilot study was performed on 3 knees (2 left and 1 right) from 3 bovines and the trends in properties across the knee surface observed are in agreement with those reported this study. The study presented here represents data from a further 6 knees from three individuals (both left and right) of bovines (all < 18 months old) and the same again of equines (18 months, mid teens and late teens). Sample preparation occurred no longer than 12 hours after slaughter. Tibial plateaus were inspected at time of dissection and no signs of osteoarthritis were found. Subsequently 8 mm punch biopsies were taken from 6 respective locations on the upper surface of the tibia (medial, lateral and anterior, middle, posterior, Fig. 1). Punches were stored overnight in DMEM at 4°C in order to ensure the tissue was still viable when tested. The following day the 8 mm punch biopsies were sub-sampled into two 3 mm punch biopsies, one was used for this study and then other reserved for future work.

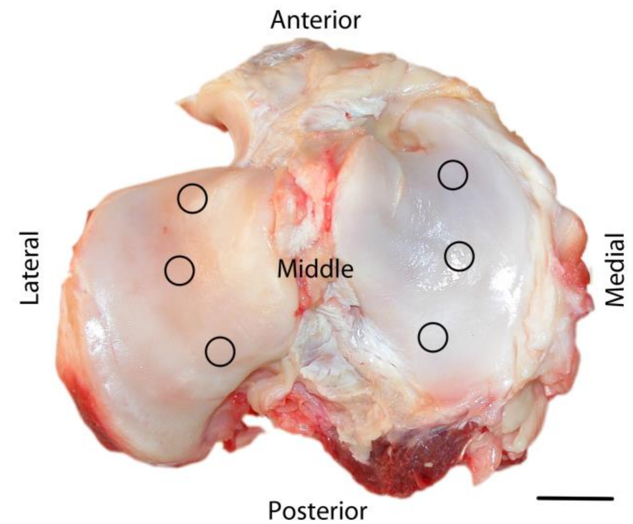

Fig. 1 Diagram depicting the location of the punch biopsies on the tibial articular cartilage used in this study. Bovine sample. Scale bar is 20mm.

Rheological testing

A Bohlin Gemini 200 HR Nano rheometer (torque range 3 nNm to 200 mNm, controlled stress/strain oscillation, Malvern Instruments, UK) was used to collect all data. Samples were loaded between two parallel plates with cartilage upper surface facing the upper plate of the rheometer and surrounded by fresh DMEM kept at 37^{o}C by an environmental chamber. In order to maintain a constant grip on the samples a 0.2 N compressive load was applied during testing. Prior to characterisation, samples had their linear viscoelastic region characterised and subsequent tests were made within this region of strain. An oscillatory test (12.5-0.1 Hz, 0.001 strain) was undertaken and valid shear modulus (G*) readings taken (as defined by a raw phase angle being within machine limits) and averaged due to the frequency independent nature of the sample moduli over the tested frequency range. The test-retest variation of DSA has been previously reported (Chaudhury et al. 2011) and validated in this study to be >1% (n=1, data not shown).

Statistical Analysis

Because the distribution of the data was not normal, non-parametric statistics were performed where $p<0.05$ was regarded as significant. Mann-Whitney U tests were performed on data to determine differences between species, sides and site-to-site comparisons. Kruskal-Wallis tests were performed within a side. The statistical package used for analysis was PASW (v.18, PASW Inc., Chicago, IL).

Results

Overall the material properties tested in this study showed highly significant differences between bovine and equine samples (Mann-Whitney U test $p < 0.0001$ for all) and when comparing the medial and the lateral sides between each species (Mann-Whitney U test $p < 0.0001$ for all except shear modulus on the lateral side where $p = 0.009$). Compared to bovine material, the equine tibial cartilage was approximately 50% thicker, 5 times less compressible and considerably more resistant to shearing forces (Fig. 2); equine cartilage thus would require significantly more energy to deform.

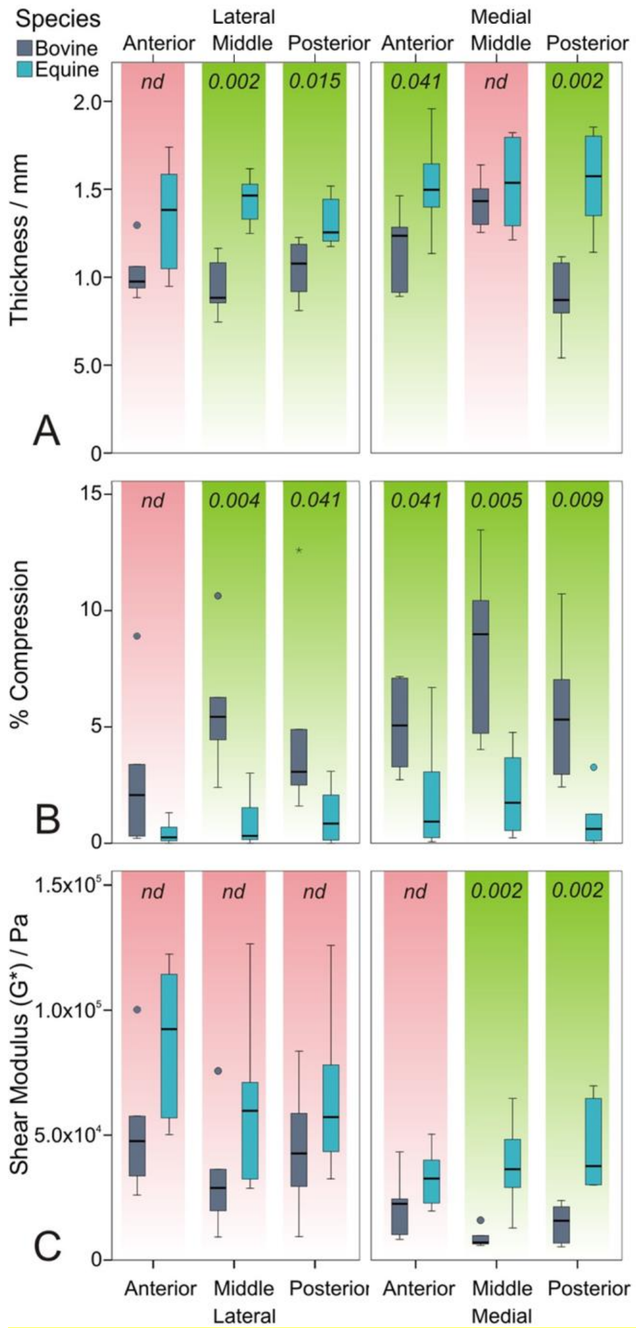

Fig. 2 The mechanical properties of bovine and equine knee cartilage as determined by dynamic shear analysis. A) Thickness, B) Compression during testing and C) Shear modulus for bovine (n=6 knees, dark-blue) and equine (n=6 knees, light-blue) tibial articular cartilage samples. Data presented as box charts indicating median, 25 and 75 percentile range and whiskers containing 95 percent range. Circles are outliers whereas stars are extreme outliers. Also shown are the p values (Mann-Whitney U) comparing

bovine to equine at each site, nd=no significant difference

In addition we detected some with-in species differences in material properties according to location in the joint. The medial and lateral sides in the bovines differed significantly in thickness, unconfined compression and shear modulus (Mann-Whitney U $p = 0.042$, 0.036 and <0.001 respectively) whereas they differed only in the shear modulus in the equines (Mann-Whitney U $p=0.001$). However, only the bovine medial side displayed significant differences between the anterior, middle and posterior positions (thickness, Kruskal-Wallis $p= 0.06$ and shear modulus, Kruskal-Wallis $p = 0.042$). Taken together and seen in the light of the overall shape and biomechanical constraints of the knees, these data strongly suggest that there may be specialisation of the joint in these areas. There were measurable differences in the shear moduli at all locations in the bovine samples (Mann-Whitney U Test anterior $p=0.015$, middle $p=0.008$, posterior $p=0.026$) as well as across the equine anterior section of the tibia (Mann-Whitney U Test $p=0.004$). Hence, it appears that cartilage material properties differ most in the medial side and the posterior section of the tibial surface suggesting these to be sites of knee specialisation (Fig. 2).

Furthermore the loading history of the joint will most likely have contributed to the variation seen in the samples. The horses used in this study were from a range of different backgrounds and ages. The youngest equine sample (18 months) displayed a 5 fold increase in compression of the cartilage and two thirds of the shear modulus when compared to the older equine samples, despite having the same thickness of cartilage. This is consistent with an age related increase of non-enzymatic crosslinking of equine collagen which has been predicted to give rise to stiffer, less compressible material (Brommer et al. 2003; Brama et al. 1999). However bovines are low impact grazers which are all slaughtered at approximately the same age. This would explain why we saw a smaller degree of inter-sample variation when compared to equines. Hence our rheometric approach also appears to be well suited for investigating developmental changes, or the effect of different lifestyles on the material properties of cartilage.

Discussion

Our study using DSA showed significant differences in the posterior sections of bovine and equine tibial cartilage with the equine cartilage being thicker, compressing less and having a greater resistance to deformation. These features support the thesis of specialisation associated with gait such as e.g. faster top and trot speeds in horses compared to cows (10.4 vs. 6.4 and 5 vs. 3.8 ms^{-1} resp. at comparable body weights) (Taylor 1985) with the argument that faster gaits result in larger forces, which must be accommodated by the storage and recovery of the associated elastic strain energy (Warner et al. 2013). Moreover, when analysing our samples for age differences we observed further details, suggesting further powers of analysis.

Breeding, as well as natural selection, has contributed to both conformation and gait of the horses and cows studied. Moreover, we must assume that all joints, as well as the bones and indeed the hooves, of these two taxa have evolved since the mid-Miocene period as units; and with ~20M years of independent evolution for each system and thousands of years of selective breeding it is not surprising that most morphological and anatomical features differ in detail (and to various degrees) between the taxa, species and races despite many the overall similarities (Southwood 2003; Pough et al. 1989). Hence it would be impertinent of us to speculate here any further on our findings other than to reiterate the apparent power of the technique used, which provides a novel tool to researchers whether there are interested in the evolution and behaviour of quadrupeds or investigating leg/foot diseases and related joint modifications.

Our observations have implications for the study of human knee cartilage and its diseases. In particular, osteoarthritis (OA) of the knee displays highly repeatable patterns. Anteriomedial OA was first described by (White et al. 1991) and the patterns of cartilage damage in both isolated medial and lateral compartment OA of the knee was later described by (Gulati et al. 2009). These damage patterns are intriguing; in isolated medial OA the cartilage damage is observed on the medial side at the front of the joint, whilst those for isolated lateral OA are observed on the lateral side toward the back of the joint. Humans have evolved from a quadrupedal to a bipedal gait and hence the common observation of repeatable but different cartilage lesion patterns in isolated medial and lateral osteoarthritis in human knees might then be a result of evolutionary drag in the material properties of our knee cartilage. The findings of the current study demonstrate that cartilage material properties differ with location going from the front of the tibial plateau to the back, and the patterns of material property variation are different between bovine and equine samples. The requirements of bi-pedal gait necessitate a drammatic increase in the range of motion of the knee compared to quadrapedal locomotion, thus exposing the posterior part of the joint to functional loading. Evolutionary pressures driving increased knee range of motion may not have driven a significant change in cartilage material properties.

In conclusion our findings in this study, combined with a recent quantitative validation of DSA in rotator cuff tendon pathology (Chaudhury et al. 2011), suggest that the technique of DSA could offer a new, powerful means to precisely quantify the pathology of osteoarthritis for medical research (although not as a diagnostic in this current guise). This potentially offers a new approach to classification where previous methods have relied mostly upon visual inspection (Gulati et al. 2009).

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Author Contributions

CH and HSG performed the experiments. All authors contributed to manuscript preparation and writing.

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